The Genetic Diversity of the Rare Idaho Endemic *Allium aaseae* Ownbey (Alliaceae) and Potential Introgression with *A. simillimum* Henderson

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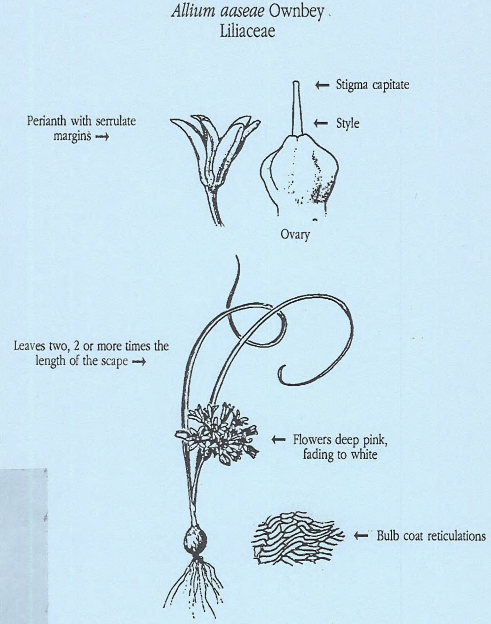

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THE GENETIC DIVERSITY OF THE RARE IDAHO ENDEMIC *ALLIUM AASEAE* OWNBEY(ALLIACEAE) AND POTENTIAL INTROGRESSION WITH *A. SIMILLIMUM* HENDERSON

FINAL REPORT

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ABSTRACT

Although recent investigations of *Allium aaseae* have confirmed its species status and identified potentially reliable characters for field identification, there have been no studies to examine genetic relatedness between *A. aaseae* and *A. simillimum*, and to confirm that the morphological characters suggested can be attributed exclusively to one species. In addition, potential genetic introgression between these two species may be obscuring the genetic integrity of *A. aaseae*. This project investigates the genetic basis for the separation of these two species using RAPD data. The data indicate that the genetic diversity of the populations examined in this study is comparable to other studies although the distribution of the genetic diversity is widespread among the populations and is not explained as diversity within populations. These results imply that either introgression between *A. aaseae* and *A. simillimum* is occurring or that *A. aaseae* is only recently derived from *A. simillimum*. An examination of phenetic relationships of the populations based on the RAPD data compared to morphological characters and geographic distribution indicate that perhaps both a recent speciation event and hybridization explain the pattern of genetic diversity found within this study. Some management considerations for *A. aaseae* are discussed in light of the fact that hybridization is likely occurring between the rare and more widespread species.

INTRODUCTION

Allium aaseae is a rare Idaho endemic found along the Boise Front. Its current known distribution is from Freezeout Hill south to Boise's Military Reserve Park (Fig. 1). One disjunct population at Rebecca Sand Hill east of Weiser has been confirmed recently as *A. aaseae* (McNeal 1993) as have other populations in Cartwright Canyon (Fig. 1, Table 1). This rare species appears to be restricted to the deep, coarse lacustrine soils of the Glenns Ferry Formation (Packard 1979) or at least coarse sandy soils along the Boise Front, normally occurring on steep slopes, or in flats below and immediately adjacent to slopes. Exposure is normally south or southwest. All populations occur from elevations between 2700 and 5100 feet, but more commonly below 3700 feet.

Allium aaseae is similar to *A. simillimum*, a more common species also found along the Boise Front, but generally occurring at higher elevations than *A. aaseae* (over 4200 feet) and with a much more widespread distribution. It is generally found on shallow granitic soils underlain by bedrock. However, it is important to note that the Cartwright Canyon population (*A. aaseae*) is also underlain with bedrock. Slope and exposure are various. Morphologically the two species are very similar and from preliminary cladistic analyses of morphological data are clearly sister species (Smith unpublished results). A similar analysis performed on flavonoid data (Mingrone 1968) does not indicate a sister species relationship between *A. aaseae* and *A. simillimum*. Instead, these results indicate that *A. aaseae* is more closely related to a group of species that includes *A. lemmonii* and *A. parvum* (Smith unpublished results). The diagnostic morphological characters for these two species have posed numerous problems for field botanists and others trying to determine the identity of these species. Most keys rely on flower color and the presence or absence of crests on the ovary to distinguish between these species (Cronquist et al. 1977; Hitchcock & Cronquist 1973; Hitchcock et al. 1969; Mingrone 1968). Other characters that have been examined include scape length (Packard 1979), pollen and anther color, and tepal

denticulations (McNeal 1993).

The traditional characters of flower color and ovary crests have proven to be unsatisfactory to distinguish between these species. Flower color is often regarded with skepticism by taxonomists, especially when comparing white to colored flowers. This character can readily be altered by a single gene and variation in flower color within a species is a common occurrence. Most members of the *Allium falcifolium* H. & A. alliance (including both *A. aaseae* and *A. simillimum*; Saghir 1966) generally show a one or two lobed crest on each ovary lobe near the base of the style. These are sometimes very small and disappear as the ovary matures, but nonetheless are present at some time in all species making this character useless to distinguish between the two species in question (McNeal 1993).

The scape length of *Allium aaseae* is statistically longer than that of *A. simillimum* (Packard 1979), but there is enough overlap in this character that it would be extremely difficult to use in the field to distinguish between the two species (McNeal 1993).

A recently examined set of characters is pollen and anther color. McNeal (1993) examined these two characters while in the field and from herbarium material. He found that this character had been misinterpreted in the descriptive work on *Allium aaseae* and *A. simillimum* (Mingrone 1968) and may actually be one of the more useful characters to distinguish the two species. All specimens of *A. aaseae* examined by McNeal (1993) had yellow anthers with yellow pollen whereas *A. simillimum* had purple, or white and purple mottled anthers and white-grey pollen. These characters are easily recognized in the field and can even be recognized on older herbarium specimens.

Lastly, tepal denticulations may not prove to be taxonomically useful, but may provide evidence for introgression between *Allium aaseae* and *A. simillimum*. The presence and distribution of denticulations on the tepals of *A. aaseae* are quite variable. Populations from Bogus Basin Road and Cartwright Canyon closely resemble the denticulations of *A. simillimum* (McNeal 1993). These two populations also represent some of the geographically closest populations of *A.*

aaseae to *A. simillimum*. Populations of *A. aaseae* that are further from *A. simillimum* have fewer denticulations (Ada Co. landfill, Boise Military Reserve Park) and the Rebecca Sand Hill Research Natural Area which is furthest removed from *A. simillimum* completely lacks denticulations (McNeal 1993). Because of the variable nature of this character it obviously lacks utility as a distinguishing character between the two species, however, the distribution of this character may indicate introgression between *A. aaseae* and *A. simillimum*. Tepal denticulations were recorded as presence or absence for all individuals in this analysis as possible indicators of hybridization between these two species.

Although recent investigations have determined that *Allium aaseae* is a distinct and valid species from *A. simillimum* (McNeal 1993), and characters have been identified to separate the species (McNeal 1993), there has been no genetic evidence that the two species are distinct and that the morphological characters proposed by McNeal (1993) are unique to species of *A. aaseae*. Likewise it is unknown if hybridization is occurring, or has historically occurred between *A. aaseae* and *A. simillimum*, or if *A. aaseae* represents a stable hybrid between *A. simillimum* and *A. tolmiei* J. G. Baker as noted by Packard (1979). Therefore, this study investigates these questions using random amplified polymorphic DNA (RAPD) technology (Williams et al. 1990).

RAPD technology has fast become a means of investigating genetic diversity within and between populations as well as a means of detecting interspecific hybridization (Xiong et al. 1991; Arnold 1993; Cruzan and Arnold 1994; Russell et al. 1993; Dawson et al. 1993; Hadrys et al. 1993; Williams and St. Clair 1993; McCoy and Echt 1993; Kazan et al. 1993; van Buren et al. 1994; Smith et al. 1995). RAPD technology relies on the technology developed for the polymerase chain reaction (PCR) but uses random primers rather than primers for specific genes.

PCR technology enables the synthesis of specific genes or loci such that the specific gene can be visualized on a gel without using elaborate visualization techniques such as autoradiography, making the work more affordable and safer. The initial steps of PCR are to mix a buffered solution that contains total genomic DNA of the organism being studied (template

DNA), appropriate concentrations of nucleotides, and DNA polymerase, the enzyme responsible for synthesizing DNA from nucleotides. The mixture also contains primers. Primers are small pieces of single-stranded DNA that range from 10-30 nucleotides in length. For most PCR reactions there are two different primers, one for each end of the gene in question. The two primers are identical or nearly identical to the sequence of nucloetides in the first 10-30 and last 10-30 nucleotides of the gene. Once the mixture is completed, the reaction mixture is cycled through three different phases or steps a specified number of times (30-60 repetitions).

The mixture is first heated to 95-97 ° C, a temperature that ensures that all of the DNA in the solutions will become single-stranded, including the template DNA. The mixture is then rapidly cooled to a lower temperature, usually around 35-58 ° C. This is termed the annealing phase and the goal is for the single-stranded primers to anneal to the beginnings and ends of the target genes in the template DNA, thus creating short regions of double-stranded DNA. It is also hoped at this point that only smaller portions, or none, of the template DNA will reanneal to itself. The third phase of the cycling is synthesis. For this final stage the temperature is brought back up to 72 ° C, the ideal temperature for the DNA polymerase used in the mixture. Since DNA polymerase will recognize only regions of double stranded DNA to begin synthesis, it will synthesize only those regions where the primers have annealed to the template DNA. These three phases are repeated 30-60 times. At the end of the cycling the target gene has been synthesized repeatedly and may have millions of copies in the reaction mixture where there was initially only a single copy. Thus, this gene would be visible when an aliquot of the reaction mixture was run on a gel and stained with ethidium bromide and viewed under UV light. The remainder of the template DNA is in such a low concentration that it can not be seen against the millions of copies of the target gene.

RAPD technology also uses the same principles and methods developed for PCR, but rather than using two specific primers that match the beginning and ends of a target gene, only a single primer is added to the reaction mixture. This primer is short (usually only 10 nucleotides

long) and is not designed to correspond to any specific gene in the template DNA. Thus it is random where annealing will occur. The synthesis will only produce a product in regions where the primer anneals close enough to another region in the template DNA where it anneals, one site acting as the beginning of a locus and the other acting as the end. Because of the random nature of the primers, and their small size, high amounts of variability can be detected at the molecular level between and within populations.

By sampling within several populations of *Allium aaseae* and *A. simillimum* as well as several individuals of *A. madidum*, *A. fibrillum*, *A. brandegei*, *A. tolmiei*, and *A. parvum*, RAPD technology has been used to 1) assess the level of genetic variation within and between populations of *A. aaseae* and *A. simillimum*, 2) determine the genetic relatedness of *A. aaseae* from *A. simillimum*, 3) use the genetic distinctions to correlate with morphological features of the different populations, and 4) compare the variation within *A. aaseae* to other *Allium* species mentioned above to determine potential origins of *A. aaseae*.

METHODS

Plant collection - Seventeen populations of *Allium aaseae* were sampled as a part of this study including the Rebecca Sand Hill and Cartwright Canyon populations, as well as several populations that morphologically were determined to be *Allium simillimum* based on anther and pollen color (Table 1). Twenty-five individuals were sampled randomly from each population. Data collected for each individual included tepal denticulations, tepal color, aboveground scape length, anther color, pollen color, and the presence or absence of crests on the ovary (appendix 1). One, and sometimes two, leaves were collected for each individual for the molecular analysis. An additional three to nine individuals per population were collected as vouchers (Table 1) and the bulb depth was recorded for these individuals. The average depth for each population is reported in Table 2.

Additionally, populations of *A. brandegei, A. tolmiei, A. fibrillum, A. madidum*, and a population of *A. simillimum* from Owyhee county were sampled (Table 1).

Data Collection - Total DNA was extracted from each individual via the CTAB method (Smith et al. 1992). This method is quick and yields high quality DNA for further studies. Once extracted, DNA was subjected to the polymerase chain reaction (PCR) using RAPD markers. Five different RAPD primer kits, consisting of 20 primers each were sampled for this analysis (Operon Technologies). Initial surveys were performed by using one individual from 15 of the sampled populations. These were then amplified using the 100 primers and the degree of utility of each primer was assessed based on the quality of the amplified products and the amount of variation seen. Once the survey was complete, fourteen populations were sampled for further analysis (Table 1).

The amplification regime was performed with 45 cycles of 1 minute at 97 ° C, 1 minute at 35 ° C, and 2 minutes at 72 ° C. The amplified products were electrophoresed in 1.4% agarose gels to separate amplified bands based on size differences. Gels were stained with ethidium bromide and visualized under UV light. Bands were scored based on size, each amplified band/primer being assigned a specific size (appendix 2). All individuals were then scored for the presence or absence of that band. These data were then transformed into allele frequencies under that assumption that each amplified band represented an allele (appendix 3).

DATA ANALYSIS

Genetic Distance - Allele frequencies were used to calculate genetic distance between all populations (Table 3) using the GENDIST program of PHYLIP (Felsenstein 1993). The resulting distance matrix was then used to construct an unrooted phenetic tree of the different populations (Figure 2) using the FITCH program of PHYLIP (Felsenstein 1993).

Genetic Diversity - To assess the overall distribution of variability between and within

populations, gene diversity statistics (Nei 1973) were calculated for each RAPD marker (Table 5) using the computer program BIOSYS-1 (Swofford and Selander 1981) using the algorithms HIERARCHY and WRIGHT78. Because of the limitations to the number of loci that this program can handle (60 maximum) ten loci were excluded from the analysis of genetic diversity values. These loci are all loci for the primers V5 and I19 as well as loci V6-1200, O18-1600, and O18-700. These loci were selected for exclusion due to identity between all populations (V6-1200, O18-700) or a higher proportion of missing data for individuals within each population (appendix 2). The total observed genetic diversity estimates (H_T) were partitioned into within population diversity (H_S) and between population diversity (D_{ST}) components. The diversity between populations was expressed as a proportion of the total diversity ($G_{ST} = D_{ST}/H_T$). The proportion of markers that were polymorphic for each population were also calculated (Table 6).

Morphological Characters - During plant collection, each individual of *Allium aaseae* and *A. simillimum* were kept separately and data on the morphological features of each individual were examined (appendix 1). These morphological data were summarized and averaged for each population. Denticulations on the tepals were calculated as the percentage of individuals in each population that had denticulations. Tepal, anther, and pollen color were averaged for each population by assigning a numerical value to each color. These numbers were then summed and averaged for each population. Tepal colors were classified as white (0), pale pink (1), pink (2), dark pink (3). Anther color was classified as yellow (2), yellow and purple (1.5) or purple (1). Pollen was either yellow (1) or white (0). Average morphological values are summarized in Table 2. Ovary crests were not encountered in any of the populations sampled. This is likely due to the early stage in development of these plants. Most populations were sampled at the time that anthesis was just beginning.

Average morphological values were then mapped onto the unrooted phenetic tree based on genetic distances (Figs. 3-8).

Identification of hybrids between *Allium aaseae* and *A. simillimum* - Because of the lack of RAPD genetic markers between these two species identification of specific individuals as interspecific hybrids was not possible (see results). However, the phenetic distance analysis, when compared with geographic distribution of populations indicates that at least some of the populations are likely to be involved in interspecific hybridization (see discussion).

Evolutionary origin of *Allium aaseae* - Packard (1979) noted that *Allium aaseae* may represent a stabilized hybrid between *A. simillimum* and *A. tolmiei*. McNeal (1993) believes that *A. aaseae* and *A. simillimum* may both share a common ancestor from the *A. fibrillum/A. madidum* complex. The same RAPD markers as well as additional primers that were monomorphic within the larger survey (see results) were used to examine the variation in individuals of *A. aaseae* (CH, RSH) *A. brandegei, A. tolmiei, A. madidum, A. fibrillum, A. parvum*, and *A. simillimum* (AS and from Owyhee county) (Table 1). The data are presented in appendix 4. These bands also were scored based on size and presence or absence per individual, but instead of analyzed as gene frequencies these markers were analyzed cladistically to better estimate evolutionary relationships. The data were entered in the computer program PAUP (phylogenetic analysis using parsimony) version 3.1.1 (Swofford 1993) and were analyzed using the exhaustive search option. *Allium parvum* was designated as the outgroup in this analysis.

RESULTS

Primer Utility - One hundred primers were surveyed for this analysis using fifteen different individuals, each from a different population. Twelve of these primers were found to be suitable for the level of investigation and are listed in Table 4. The remaining 88 primers were determined to be monomorphic or nearly monomorphic across all individuals surveyed (32), produced poor or no amplification products (46), or produced an excessive number of bands (10).

Loci - The twelve primers resulted in 67 different loci scored across all 350 individuals in

fourteen different populations. These are listed in appendix 2. Two of these loci were found to be monomorphic across all individuals sampled (V6-1200, O18-700). These data were used to calculate the frequency of each locus among the individuals for all populations (appendix 3) which were then used to determine pairwise the genetic distance between all populations (Table 3) and ultimately to construct an unrooted phenetic tree (Figure 2).

Morphology - Six morphological features were measured for all individuals in this analysis and the data are summarized in appendix 1 except for ovary crests which were not present in any of the individuals at the time of sampling. Average values for each of these features were calculated for each population including bulb depth which was determined using an additional three to nine individuals from each population. Average values for the presence of denticulations on the tepals was calculated as a percentage of individuals with denticulations. Average color values were determined by assigning a numerical value to the different color classes. For the tepals four different color classes were used; white (0), pale pink (1), pink (2), and dark pink (3). The anthers were divided into three color classes, yellow (2), yellow and purple (1.5), and purple (1). Pollen was classified into two color categories, yellow (1) and white (0). Averages for all morphological features are summarized in Table 2.

The average for each morphological feature for each population was mapped onto the unrooted phenetic tree (Figure 2) to demonstrate the distribution of each morphological feature. The average value for each population is presented numerically as well as symbolically for each feature. These are presented in Figures 3-8.

Genetic Diversity - The RAPD data for the populations of *Allium* sampled in this study indicate low to comparable levels of genetic diversity when compared with other species where RAPD data have been used (Dawson et al. 1993; Russell et al. 1993). Total genetic diversity (H_T) ranged from 0.035-0.500 with a mean of 0.343 (Table 5). Within (H_S) and between (D_{ST}) population diversity components ranged from 0.035-0.448 and 0.000-0.172, respectively (Table 5). The means for within and between population diversity were 0.282 and 0.052 respectively (Table 5).

These data imply that the total gene diversity of these *Allium* populations is more likely to be found among individuals than between populations. The proportion of the total genetic diversity that is attributed to between population variation (G_{ST}) ranged from 0.008-0.372 with a mean of 0.136 (Table 5). The proportion of polymorphic loci per population ranged from 0.403 (RSH) to 0.672 (BC) and are presented in Table 6.

Evolutionary Origin of *Allium aaseae* - The RAPD data for the different species of *Allium* used in this analysis (Table 1) yielded four most-parsimonious trees of thirty-four steps each based on the exhaustive search option of PAUP (Figure 9 A-D). The strict consensus of these four trees is presented in Figure 10.

DISCUSSION

Genetic variation has been assessed in the rare Idaho endemic, *Allium aaseae* using RAPD data. RAPD data in general have demonstrated a substantial amount of genetic variation in the populations of *A. aaseae* and *A. simillimum* that were sampled in this analysis (Table 1). One hundred RAPD primers were surveyed for this study and 12 were determined to provide the amount of variation suitable for the level of analysis in this investigation. The 12 primers produced 67 loci of which only two were found to be monomorphic. In comparison to other analyses where RAPD data have been used to assess genetic diversity, the diversity found within *A. aaseae* is comparable or slightly lower. Dawson et al. (1993) sampled 10 populations of *Hordeum spontaneum* in Israel using five individuals per population which were then surveyed with ten RAPD primers. Thirty-six loci were reported from their investigation. This is approximately half the number of loci found for *Allium*, however, ten instead of 14 populations were surveyed and approximately 50 rather than 350 individuals were examined.

Similarly a study on *Theobroma cacao*, using 25 individuals in six populations and surveying nine RAPD primers found a reported 75 loci (Russell et al. 1993). This is a greater

amount of variation with a substantially smaller sample size.

In general these data indicate that the overall genetic variation within *Allium* (both *A. aaseae* and *A. simillimum*) are slightly lower than other plants, a trend that has been seen with other rare plants using isozyme variability (Karron 1991). Karron (1991) made a comparison of genetic diversity using geographically restricted versus widespread congeners based on isozyme data. In this review restricted species generally had fewer polymorphic loci than their more widespread congeners. However the ranges are quite broad with the percentage of polymorphic loci for restricted species ranging from 3.4-90.5% and the widespread species ranging from 0.0-94.7%.

Allium aaseae and *A. simillimum* also fit into this pattern although there is a clear overlap between the range of genetic polymorphism for the two species. The percentage of polymorphic loci for populations of *A. aaseae* range from 40.3-62.7% and for *A. simillimum* from 52.2-67.2% (Table 6). This indicates that *A. aaseae*, the restricted species, has a lesser degree of gene diversity per locus than does the more widespread *A. simillimum*. Although there is a clear overlap in the ranges of polymorphism between the two species, the population with the lowest percentage of polymorphic loci is one of *A. aaseae* (RSH, the most remote population) and the highest is one of *A. simillimum* (BC).

These polymorphic loci data also indicate that no single population is likely to carry a quantity of the gene diversity found among these populations than is any other population. In addition, when compared to other investigations using allozyme data on species where the breeding system is known, high levels of polymorphism, as seen in this study indicate high levels of outcrossing among individuals and between populations (Hamrick and Godt 1990).

Geographic range is only a single parameter in the life history of plants and many other factors may effect the pattern of genetic diversity. Hamrick et al. (1991) review correlations between life history traits and allozyme diversity in plants with the conclusion that geographic distribution produces no significant differences in the levels of genetic diversity among populations, although widespread species generally have high levels of genetic diversity at the

population level. However several other characteristics of species with high within and low between population diversity such as seen with *Allium* are: monocots, long-lived perennials, and temperate species (Hamrick et al. 1991). An additional parameter regarding the breeding system shows a correlation between wind-pollination (high levels of genetic diversity at the population level and low levels of genetic diversity among populations) and the genetic diversity seen with *Allium* in this study. The pollination biology of neither *A. aaseae* nor *A. simillimum* is known at this point and would provide valuable information to confirm the findings of this study regarding the levels of genetic diversity.

A comparison of genetic diversity values (Nei 1973, Table 5) demonstrate that for a comparable study where the same values were calculated using RAPD data (Dawson et al. 1993) *Allium* has a slightly higher overall diversity value (H_T) with an average of 0.343 vs an average of 0.257 for *Hordeum* (Dawson et al. 1993). It should be emphasized here that the values for *Hordeum* are based on a single species (*H. spontaneum*) as well as 10 populations and 36 loci compared to two species of *Allium* with 14 populations and 57 loci. Therefore it is not surprising to find slightly higher genetic diversity values for *Allium*.

A more interesting comparison is the value for the proportion of the total genetic diversity that can be explained as variation between populations (G_{ST}). In other words, how distinct is each population genetically from other populations. For *Hordeum* this value is an average of 0.433 (Dawson et al. 1993) and for *Allium* it is 0.136. This substantially lower value for *Allium* indicates that although the total genetic diversity for the populations examined in this study may be relatively high, that diversity is more equally proportioned throughout its range and is not partitioned among the populations examined in this investigation. No single population examined in this study contains a substantial amount of genetic information that is unique to that population. A caution should be made in interpreting these data in saying that there are no unique markers among the populations of *Allium* examined in this study. There are several markers that are restricted to one or few populations (appendix 2). However, the markers that were examined in

this analysis are overall found in most populations and not restricted to any single population.

The low value for G_{ST} found with the *Allium* populations examined in this study could be explained by two mechanisms. 1) widespread gene flow among all populations examined in this analysis, or 2) *A. aaseae* is only recently derived from *A. simillimum*. Both scenarios are possible and it may be that a combination of both can explain the patterns of diversity seen in this study.

Gene flow among the populations, and between the two species is likely as shown by the genetic relatedness of the populations between species (Figure 2). This is especially true for the pairwise groupings of some populations of *Allium aaseae* (CH, LHG, and SG) with geographically close populations of *A. simillimum* (UHG, AS, and DC) (Figures 1 & 2) (see discussion below). The low level of polymorphism seen for the most remote population of *Allium* used in this study, RSH (Figure 1) also supports the hypothesis of substantial gene flow. The more geographically clustered populations have higher levels of polymorphic loci, indicative of gene flow due to proximity. Since RSH is isolated it would be less likely to interbreed with other populations and thus, alleles would become fixed at a faster rate.

It is also possible that the genetic relatedness between *Allium aaseae* and *A. simillimum* may reflect a recent separation of the two species and that substantial numbers of unique genetic markers have not had time to evolve within *A. aaseae*. A similar scenario has been hypothesized for two species of *Layia* based on isozyme data. *Layia discoidea* is a serpentine endemic restricted to 155 km^2 of the inner South Coast Range of California and has the highest percentage of polymorphic loci (90.5%) for a geographically restricted species as reviewed by Karron (1991). This species is morphologically distinct from its widespread relative, *L. glandulosa*, but the two are very similar genetically as based on isozyme data (Gottlieb et al. 1985). These data were used to support the conclusion that the restricted serpentine endemic, *L. discoidea*, was recently derived from its more widespread congener, *L. glandulosa* (Clausen et al. 1947; Gottlieb et al. 1985). The similar pattern seen for the *A. aaseae/A. simillimum* pair suggests that a similar scenario may explain the pattern of genetic and morphological diversity of the two species (see below).

Genetic relationships among populations of *Allium* in the Boise Front - The results of this analysis do not indicate a clear genetic distinction between populations of *Allium aaseae* and *A. simillimum* in the Boise Front (Figures 2-8; Table 5). Populations that were identified as *A. simillimum* based on several morphological characters are not distinctly separated in these analyses (Figures 2-8) although there is a general trend in the tree. There are several possible explanations for these results.

1) The populations sampled in this study are all members of *Allium aaseae* and no true members of *A. simillimum* were sampled. The populations from Danskin Mountain (LDM) Black's Creek (BC), and Long Gulch (BF) were purposefully included in this analysis as certain representatives of *A. simillimum*. The individuals were in general smaller, and occurred at higher elevations than is typical for *A. aaseae*. The individuals in these three populations were also clearly different in their morphological characters in having lighter colored flowers, purple anthers, shorter flowering scapes, shallower bulb depths, and white pollen (Table 2, Figures 3-8). However, the identity of these populations have drawn speculation previously but were concluded to be members of *A. simillimum* and not *A. aaseae* (Mancuso and Moseley 1991). Despite these data, the genetic analysis clearly places the population Long Gulch (BF) with Willow Hollow (WH), Rebecca Sand Hill (RSH), and Collister Hillside (CH) and very distant from both Lower Danskin Mountain (LDM) and Black's Creek (BC), the latter two showing an expected close relationship (Figure 2). This question can only be resolved by sampling populations of *A. simillimum* that are geographically distant from the populations sampled in this analysis.

2) It is possible that insufficient time has lapsed since the genetic separation of *Allium aaseae* from *A. simillimum* and as a result the two species still share many of the same alleles in approximately the same frequencies. The pattern of relationships seen in the distance analysis may indicate a lack of divergence time between the two species. This is mainly because there is not a complete correlation between the geographic distribution of the populations and their respective distribution in the tree (Figures 1& 2, however see below). A stronger correlation would be more

indicative of interspecific hybridization, although numerous pairs may indicate that interspecific hybridization is occurring.

There is a strong likelihood that *A. aaseae* and *A. simillimum* are sister species based on cladistic analysis of morphology (Smith unpublished results) and RAPD data (see below). The likelihood that *A. aaseae* is derived from within a species of *Allium* other than *A. simillimum*, or hybridization with *A. simillimum* and another species is low (see below). Thus it is possible that *A. aaseae* is derived from recently isolated populations of *A. simillimum* and may therefore not demonstrate distinct genetic divergence at this time.

3) It is possible that there is genetic introgression between the populations of *Allium aaseae* and *A. simillimum*. Interspecific hybridization would most likely reflect some degree of geographic correlation, e.g., populations that are geographically close would be more likely to be involved in interspecific hybridization than more distant populations and would therefore be expected to be more similar genetically. Although this is not absolute in this analysis, many of the populations from the two species that are geographically close, are also genetically close. This is true for the following pairs of *A. aaseae* and *A. simillimum* with the *A. aaseae* population listed first: Lower Hull's Gulch (LHG) & Aldape Summit (AS), Collister Hillside (CH) & Upper Hull's Gulch (UHG), and Seaman's Gulch (SG) & Daniel's Creek (DC) (Figures 1 & 2). There are however two discrepancies to this hypothesis. Two populations that are genetically similar are Willow Hollow (WH) and Long Gulch (BF) (Figure 2). However, these two populations are geographically very distant from each other (Figure 1). Likewise, the populations of *A. simillimum* Black's Creek (BC) and Lower Danskin Mountain (LDM) are genetically close to both Wood Gulch (WG) and Sand Hollow (SH) which are some of the most geographically distant populations used in the analysis (Figure 1).

4) Multiple origins of *Allium aaseae* may also explain the pattern of genetic diversity found in this study. If the populations of *A. aaseae* were each derived independently from each other, or at least some were derived independently from *A. simillimum* then the genetic analysis would not

be expected to group all populations of *A. aaseae* together and separated from all populations of *A. simillimum*. The pairwise groupings of Collister Hillside (CH) & Upper Hull's Gulch (UHG), Seaman's Gulch (SG) and Daniel's Creek (DC), Lower Hull's Gulch (LHG) and Aldape Summit (AS) may reflect this hypothesis, however the genetic relatedness of the more distant populations sampled in the analysis do not add evidence to this hypothesis.

5) The relatedness of the different populations may be an artefact of RAPD analysis. Although RAPD data have been used successfully in numerous other studies (Adams and Demeke 1993; Kazan et al. 1993; Williams and St. Clair 1993), and have shown strong correlations to similar studies with isozymes (Dawson et al. 1993), the nature of the loci scored in this analysis is still uncertain. It is possible that although the bands were scored as the same loci, there may be subtle length differences not detectable using the methods of this analysis, or potentially very different sequences between the primer binding sites. Thus, although the bands appeared as identical in the gels, they may be very different loci, and the relatedness between *A. aaseae* and *A. simillimum* may be an artefact of misinterpretation of the bands. This hypothesis is less likely, at least for some of the primers used in the analysis, as two populations of *A. aaseae* (Rebecca Sand Hill (RSH), Collister Hillside (CH)), two populations of *A. simillimum* (Aldape Summit (AS), Owyhee County) were compared side-by-side on the same gel with other species of *Allium*. The bands were clearly identical in size, and although there may easily have been different sequences in the DNA of the bands, the likelihood of this occurring repeatedly to skew the data is extremely low. However, there were some primers that were difficult to interpret and the unusual position of the more remote populations of *A. simillimum* (BF, BC, and LDM) may be the result of mis-scoring the bands.

Hybridization between *Allium aaseae* and *A. simillimum* - Although there may be several explanations for the genetic relationships seen in this study (see above) the most likely explanation for at least some populations is hybridization between the two species examined in this analysis. The strong correlation between genetic relatedness (Figure 2) and geographic proximity

(Figure 1) are highly indicative of hybridization between populations of the two species. Further evidence can be obtained by looking at the morphological features of the individuals that were sampled in this study, particularly those individuals from populations that appear genetically intermediate between the species (or at least are in the center of the tree, Figure 2). In general the morphological features sort the two species quite well. For example, populations that are attributed to *Allium aaseae* in general have less than 50% of the individuals with tepal denticulations, have tepals that are considered pale pink to pink (averaged numerically greater than 1.5), have yellow anthers, yellow pollen, and bulbs that are over 3.5 cm deep in the soil (Table 2, Figures 3, 4, 6-8). However these features are not exclusive to populations of *A. aaseae*, and not all populations of *A. aaseae* are described by these traits. For example, the population of *A. simillimum*, BF has fewer than 50% of its individuals with tepal denticulations, the populations, AS and DC both have bulbs that are greater than 3.5 cm below soil level, anther color is intermediate between the two species in AS, DC, and UHG, and pollen color is exclusively white in only two populations of *A. simillimum*, LDM and BC. Likewise the population SG of *A. aaseae* has an average tepal color closer to pale pink (1.12 average value). The only two populations that seem to be cleanly separated based on all morphological traits are LDM and BC of *A. simillimum*. These two populations are also the most geographically isolated (Figure 1) and genetically isolated from the remainder of the populations (Figure 2) although WG of *A. aaseae* is genetically similar to LDM.

Correlation between genetic and morphological identity for *Allium aaseae* and *A. simillimum* - Throughout this study, anther, and in particular pollen color were used to distinguish between populations of *A. aaseae* and *A. simillimum* Examination of additional characters that were sampled showed that *A. simillimum* tended to have a lighter tepal color, greater frequency of denticulations on the tepals, and shallower bulbs, although these latter three traits are not diagnostic traits for individuals but for the population in general. However, when these morphological traits are compared to the genetic composition of the population there is little correspondence (Figures 2-8). As discussed above there appears to be extensive hybridization

occurring between the two species and thus many genetic, and to some extent morphological features are mixing between the two species. However, both morphological and genetic markers may prove useful beyond the hybrid zone between the two species. This is demonstrated by the greater genetic isolation of both LDM and BC. These two populations of *A. simillimum* also demonstrate the least amount of morphological introgression from *A. aaseae* of the populations examined in this study. It is interesting that although most of the populations of *A. simillimum* showed some degree of morphological introgression from *A. aaseae* there was little reverse introgression based on morphology at least for the two more diagnostic traits of anther and pollen color. There were no individuals in the populations of *A. aaseae* that did not have yellow anthers and yellow pollen. The other features of tepal color, tepal denticulations, scape length, and bulb depth show introgression in both directions.

Evolutionary origin of *Allium aaseae* - A cladistic analysis was performed on twenty-one RAPD markers for two individuals of *A. aaseae* (CH and RSH), two individuals of *A. simillimum* (AS and Owyhee county) as well as a single representative from *A. brandegei*, *A. parvum*, *A. tolmiei*, *A. fibrillum* and *A. madidum*. The results of this analysis are presented in Figures 9 A-D and 10. Packard (1979) noted that *Allium aaseae* may represent a stabilized hybrid between *A. simillimum* and *A. tolmiei*. McNeal (1993) believes that *A. aaseae* and *A. simillimum* may both share a common ancestor from the *A. fibrillum/A. madidum* complex. The cladistic analysis of RAPD markers does not necessarily exclude either of these hypotheses but unless there has been such extensive backcrossing to *A. simillimum* to the point that *A. aaseae* has lost most of the genetic material from *A. tolmiei*, it is unlikely that *A. aaseae* is a hybrid between these two species. Instead it is far more likely that *A. aaseae* is derived from within *A. simillimum* and that both are a part of the *A. fibrillum/A. madidum* complex. This conclusion is also supported based on a cladistic analysis of morphological features (Smith, unpublished results). Further investigations based on specific chloroplast (uniparentally inherited) and ribosomal (biparentally inherited) genes are underway that will hopefully resolve this issue further.

A possible scenario for the origin of *Allium aaseae* based on its genetic relatedness to *A. simillimum* is that *A. aaseae* is recently derived from *A. simillimum* probably via populations isolated during the Pleistocene. During the Pliocene, large lakes and rivers covered the areas where *A. aaseae* is found today (Kimmel 1982). These lakes left deposits known as the Glenns Ferry formation which can be dated from 6.2 to 4.4 million years old (Kimmel 1982). Since *A. aaseae* is generally restricted to these soil formations, the species can not be older than 6.2 million years and is more likely to be closer to the younger age of the formation as this would indicate that periodic flooding of the area had ceased. During the Pleistocene (20-140, 000 years ago), mountain glaciers formed at higher elevations in the mountains to the north and east of the Boise Front (Evenson et al. 1982). If *A. simillimum* occurred in these mountains at that time, the change in climate would have forced the species to move to lower elevations. Populations of *A. simillimum* would have become restricted and isolated from each other at this time. *Allium simillimum* also generally occurs on sandy soil types and the Glenns Ferry formation soils may have been some of the only sandy soils available for colonization by *A. simillimum* from higher elevations. The widespread mountain glaciers would isolate the populations of *A. simillimum* and eventually those restricted to the Glenns Ferry formation would become the distinct species *A. aaseae*. The recession of the glaciers would then allow *A. simillimum* to return to its previous range. Therefore the populations of *A. simillimum* that are found within the Boise Front may reflect more recent arrivals that have now begun to hybridize with *A. aaseae*.

Management Considerations for *Allium aaseae* - Hybridization and introgression between rare and widespread plant species has been a topic of debate for several years resulting in both harmful and beneficial consequences for the conservation of biological diversity (Cade 1983). Hybridization may lead to greater genetic diversity and increased fitness (Stebbins 1942; Anderson 1949) or could lead to the loss of genetic diversity by the assimilation of the genetic diversity of a rare species into a more common species with the possibility that the rare alleles of the rare species will subsequently be lost (Reiseberg et al. 1989; Liston et al. 1990). These raise obvious

questions for the management of rare species where hybridization occurs such as with *Allium aaseae*. Reiseberg (1991) comments that neither alleles unique to rare populations nor the populations themselves are lost as a result of interspecific hybridization although the potential for the dilution of the alleles characteristic of a rare species could lead to their subsequent loss.

Allium aaseae, unlike other comparable studies of hybridization within rare plants (Reiseberg et al. 1989), does not appear to have a substantial proportion of unique alleles. Therefore, hybridization with *A. simillimum* may not result in the dilution and loss of rare alleles (Note that the population of *A. aaseae* at CH contains several unique and rare markers (appendix 2). This population also appears to be hybridizing with *A. simillimum* and is perhaps one of the most endangered populations due to its proximity to housing developments). Although based on the genetic markers used in this analysis, the distinctions between *A. aaseae* and *A. simillimum* are not substantial, the morphological characteristics of the two species are still distinctive (Table 2). This is especially true regarding anther and pollen color (Table 2). There may be numerous other morphological, ecological, and physiological traits that have not been examined that will also discriminate between the two species. It is also possible that due to the edaphic specialization of *A. aaseae* that there may be selection against many of the alleles that mark the specific differences between *A. aaseae* and *A. simillimum* but other, non-selected alleles (such as RAPD's) may be readily exchanged and integrated between the two species.

Although Reiseberg et al. (1989) recommended the removal of the more widespread species from the range of the rare species or transplanting of the rare species to more isolated sites, neither of these solutions is recommended or practical for *Allium aaseae*. 1) *A. aaseae* is restricted to a specific soil type, and transplants will not necessarily be successful. 2) The hybridization between the two species is likely due to a natural event and not through the artificial introduction of a more widespread species.

A more important question for *Allium aaseae* is how to maintain its present levels of genetic diversity to allow for subsequent evolution? Clearly, because of the lack of genetically unique

populations within the species, there must be, or have been, widespread gene flow among the populations (Hamrick et al. 1991). An important management consideration for *A. aaseae* will be how to maintain the high levels of gene flow in the future. This is especially important as the current number and size of *A. aaseae* populations are being reduced, largely due to housing developments. It will be important in the future to determine the pollination biology of *A. aaseae* in order to make better management considerations. Since the species flowers early in the season, it may make use of any and all insect taxa that are active at that time and could be an important food source for these insects. Two types of human disturbance have been particularly detrimental to bee populations as identified by Karron (1991) and may have importance on the gene flow for *A. aaseae*. These are 1) the use of pesticides that may be harmful to all insects, and 2) livestock grazing that may result in the destruction of underground nests of many insects, in particular some species of *Bombus* that use abandoned rodent burrows (Byron 1980) that can be collapsed from livestock trampling (Sugden 1985).

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Table 1. Locality and voucher information for all populations of *Allium* examined in this analysis. Populations used in the full analysis are in bold. All voucher numbers are of J. F. Smith and are deposited at the Snake River Plains herbarium (SRP).

Species	Site Name/Abbreviation	Site Locality	Voucher
A. aaseae	**Lower Hull's Gulch/LHG**	T4N. R2E. s.25	2732
	Seaman Gulch/SG	T4N. R2E. s. 6	2733
	Military Reserve/MR	T3N. R2E. s. 2	2734
	Willow Hollow/WH	T5N. R1W. s.4	2735
	Wood's Gulch/WG	T5N. R1E. s.22	2736
	Sand Hollow/SH	T6N. R1W. s14-16	2737
	Upper Stewart Gulch/USG	T4N. R2E. s. 23	2738
	Rebecca Sand Hill/RSH	T11N. R4W. s. 29	2739
	Collister Hillside/CH	T4N. R2E. s. 20	2740
	Prospect Peak/PP	T6N. R1E. s. 24	2742
	Cartwright Canyon/CC	T6N. R2E. s. 3	2752
A. simillimum	**Upper Hull's Gulch/UHG**	T4N. R3E. s. 20	2741
	Daniel's Creek/DC	T5N. R2E. s. 36	2744
	Aldape Summit/AS	T4N. R3E. s. 35	2745
	Lower Danskin Mt./LDM	T1N. R6E. s. 13	2746
	Black's Creek/BC	T2N. R4E. s. 13	2747
	Long Gulch Road/BF	T3N. R7E. s. 31	2748
A. parvum	Mud Flat Road	T7S. R3E. s. 8	2497
A. brandegei	Shingle Flat	T17N. R1E. s. 29	2807
A. tolmiei	Middle Fork Weiser River	T15N. R1E. s. 11	2790

A. madidum	Rock Flat	T19N. R2E. s. 36	3368
A. fibrillum	Craig Mountain	T32N. R4W. s. 15	3367

Table 2. Average morphological features for the populations sampled in this study. Denticulations are the frequency of individuals in that population with denticulations. Tepal color was based by assigning values to the different colors, white = 0, pale pink = 1, pink = 2, dark pink = 3. Scape length is presented in cm. Anther and pollen color were assigned numerical values as with tepal color; anthers were yellow (2), yellow/purple (1.5), or purple (1) and pollen was either yellow (1) or white (0). Bulb depth was calculated as an average from individuals that were not part of the other sampling. Three to nine individuals were sampled from the population. Bulb depth was measured from the top of the bulb to the point were the leaves and scape emerged from the soil.

Population	Dentic.	Tepal	Scape	Anther	Pollen	Bulb
CC	0.48	1.88	1.3	2.0	1.0	3.6
BF	0.32	0.00	1.2	1.0	0.04	3.4
AS	0.68	0.16	1.3	1.52	1.0	4.1
DC	0.52	1.04	1.2	1.52	0.96	3.7
UHG	0.88	0.68	1.3	1.52	1.0	3.3
CH	0.20	1.72	1.5	2.0	1.0	6.7
RSH	0.08	1.84	1.0	2.0	1.0	6.0
SH	0.16	1.84	1.3	2.0	1.0	3.8
WG	0.12	1.56	1.1	2.0	1.0	4.7
WH	0.32	1.64	1.0	2.0	1.0	
SG	0.24	1.20	1.7	2.0	1.0	5.5
LHG	0.24	1.72	1.6	2.0	1.0	6.8
BC	0.68	0.08	1.3	1.0	0.0	2.8
LDM	0.44	0.32	1.1	1.0	0.0	2.7

MR	0.28	1.40	1.4	2.0	1.0	7.1
USG	0.08	1.80	1.6	2.0	1.0	6.4
PP	0.12	1.88	0.6	2.0	1.0	2.4

Table 3. Pairwise genetic distances for all populations in this analysis.

	SH	DC	AS	CH	WG	BC	LDM	SG	RSH	CC	BF	LHG	UHG	WH
SH	0.0000	0.1586	0.1608	0.2859	0.1193	0.1688	0.1800	0.1272	0.2449	0.1977	0.2047	0.1514	0.2283	0.1860
DC	0.1586	0.0000	0.1415	0.3626	0.1713	0.2366	0.2195	0.1367	0.3630	0.2025	0.2627	0.1526	0.2268	0.1994
AS	0.1608	0.1415	0.0000	0.2664	0.1726	0.1490	0.2352	0.0984	0.2518	0.1427	0.1825	0.0854	0.1506	0.1694
CH	0.2859	0.3626	0.2664	0.0000	0.3597	0.3037	0.3683	0.3015	0.2982	0.2759	0.3443	0.3071	0.1887	0.2702
WG	0.1193	0.1713	0.1726	0.3597	0.0000	0.1591	0.1449	0.1461	0.3119	0.2140	0.3376	0.1884	0.2781	0.2814
BC	0.1688	0.2366	0.1490	0.3037	0.1591	0.0000	0.1903	0.1700	0.3068	0.2376	0.2491	0.1772	0.2703	0.2620
LDM	0.1800	0.2195	0.2352	0.3683	0.1449	0.1903	0.0000	0.2304	0.3159	0.2914	0.3599	0.2734	0.2932	0.3366
SG	0.1272	0.1367	0.0984	0.3015	0.1461	0.1700	0.2304	0.0000	0.3109	0.1657	0.1826	0.1275	0.1726	0.1680
RSH	0.2449	0.3630	0.2518	0.2982	0.3119	0.3068	0.3159	0.3109	0.0000	0.2220	0.1949	0.2791	0.2751	0.2835
CC	0.1977	0.2025	0.1427	0.2759	0.2140	0.2376	0.2914	0.1657	0.2220	0.0000	0.2230	0.1505	0.1438	0.1666
BF	0.2047	0.2627	0.1825	0.3443	0.3376	0.2491	0.3599	0.1826	0.1949	0.2230	0.0000	0.1584	0.2601	0.1083
LHG	0.1514	0.1526	0.0854	0.3071	0.1884	0.1772	0.2734	0.1275	0.2791	0.1505	0.1584	0.0000	0.1929	0.1504
UHG	0.2283	0.2268	0.1506	0.1887	0.2781	0.2703	0.2932	0.1726	0.2751	0.1438	0.2601	0.1929	0.0000	0.1611
WH	0.1860	0.1994	0.1694	0.2702	0.2814	0.2620	0.3366	0.1680	0.2835	0.1666	0.1083	0.1504	0.1611	0.0000

Table 4. Primers used in this analysis

Primer	Sequence
AA-17	GAGCCCGACT
AA-20	TTGCCTTCGG
V-1	TGACGCATGG
V-2	AGTCACTCCC
V-5	TCCGAGAGGG
V-6	ACGCCCAGGT
O-7	CAGCACTGAC
N-3	GGTACTCCCC
I-19	AATGCGGGAG
O-18	CTCGCTATCC
N-13	AGCGTCACTC
N-15	CAGCGACTGT

Table 5. Nei's (1973) genetic diversity values for the RAPD fragments used in this study.

Fragment	H_T	H_S	D_{ST}	G_{ST}
AA17-603	0.250	0.189	0.061	0.243
AA17-650	0.409	0.358	0.051	0.125
AA17-690	0.283	0.248	0.035	0.125
AA17-872	0.459	0.422	0.037	0.081
AA17-900	0.194	0.182	0.012	0.060
AA17-1000	0.320	0.251	0.069	0.216
AA17-1078	0.490	0.392	0.098	0.201
AA17-1200	0.464	0.331	0.133	0.287
AA17-1353	0.249	0.229	0.020	0.082
AA17-1500	0.185	0.171	0.014	0.075
AA17-2100	0.035	0.035	0.000*	0.008
V6-700	0.453	0.425	0.028	0.062
V6-550	0.493	0.390	0.103	0.208
V6-500	0.499	0.386	0.113	0.227
V6-1078	0.429	0.310	0.119	0.277
V6-872	0.370	0.333	0.037	0.099
V2-1400	0.500	0.386	0.114	0.228
V2-1475	0.299	0.285	0.014	0.047
V2-650	0.431	0.318	0.114	0.264
V2-700	0.193	0.176	0.017	0.088

V2-900	0.331	0.287	0.044	0.133
V2-950	0.477	0.340	0.137	0.288
V2-1078	0.299	0.284	0.015	0.049
O7-700	0.482	0.404	0.078	0.161
O7-850	0.454	0.363	0.091	0.201
O7-600	0.499	0.401	0.098	0.239
O7-650	0.463	0.352	0.111	0.239
O18-600	0.303	0.274	0.029	0.095
O18-900	0.335	0.307	0.028	0.085
O18-950	0.190	0.182	0.008	0.042
O18-1100	0.393	0.332	0.061	0.156
O18-1380	0.104	0.101	0.003	0.031
O18-1200	0.104	0.102	0.002	0.020
O18-1078	0.257	0.229	0.028	0.108
O18-500	0.279	0.253	0.026	0.093
O18-680	0.396	0.249	0.147	0.372
N3-1078	0.332	0.300	0.032	0.096
N3-1000	0.316	0.277	0.039	0.123
N3-1400	0.500	0.421	0.079	0.157
N13-1150	0.425	0.371	0.054	0.127
N13-1000	0.387	0.356	0.031	0.081
N13-1400	0.496	0.420	0.076	0.154
N13-870	0.495	0.448	0.047	0.095
N13-620	0.496	0.393	0.103	0.207
N13-1078	0.131	0.127	0.004	0.030
N13-850	0.079	0.068	0.011	0.135

N13-1600	0.133	0.115	0.018	0.133
N13-1800	0.133	0.115	0.018	0.133
N13-660	0.046	0.045	0.001	0.014
N15-1800	0.241	0.184	0.057	0.235
N15-1200	0.410	0.382	0.028	0.068
N15-900	0.454	0.417	0.037	0.081
N15-870	0.459	0.407	0.052	0.113
N15-700	0.389	0.365	0.024	0.062
N15-600	0.210	0.197	0.013	0.061
N15-1300	0.499	0.327	0.172	0.345
N15-750	0.057	0.056	0.001	0.010
mean over all markers	0.343	0.282	0.052	0.136

*value beyond significant digits displayed = 0.00028

Table 6. Genetic variability for *Allium* populations examined in this study.

Population	Proportion of polymorphic loci
SH	0.582
DC	0.522
AS	0.567
CH	0.627
WG	0.627
BC	0.672
LDM	0.567
SG	0.627
RSH	0.403
CC	0.612
BF	0.642
LHG	0.507
UHG	0.567
WH	0.567

Figure 1. Distribution map of *Allium aaseae* (shaded area). Populations sampled in this analysis are indicated by dots and labelled with abbreviations used in Table 1 of text.

Figure 2. Phenetic tree based on genetic relatedness of *Allium* populations. CH, CC, WH, RSH, SG, SH, WG, and LHG are considered populations of *A. aaseae* based on diagnostic morphological characters whereas BF, DC, BC, LDM, AS, and UHG are considered *A. simillimum*. See Table 1 of text for population abbreviations.

Figure 3. Phenetic tree with frequency of tepal denticulations indicated for each population. The frequency reperesents the percantage of individuals in each population that had tepal denticulations. The frequencies are indicated numerically as well as by the darkness of shading in each box.

Figure 4. Phenetic tree with average scape length indicated for each population. The averages are indicated numerically as well as by the length of the line.

Figure 5. Phenetic tree with average bulb depth indicated for each population. The averages are indicated numerically as well as by the length of the line.

Figure 6. Phenetic tree with average tepal color indicated for each population. The average tepal color was determined numerically by assigning numerical values of 0 for white, 1 for pale pink, 2 for pink, and 3 for dark pink. The average colors are indicated numerically as well as by the darkness of shading in each box.

Figure 7. Phenetic tree with average anther color indicated for each population. The average anther color was determined numerically by assigning numerical values of 2 for yellow, 1.5 for purple/yellow mottled, and 1 for purple. The average colors are indicated numerically as well as

by the darkness of shading in each box.

Figure 8. Phenetic tree with average pollen color indicated for each population. The average pollen color was determined numerically by assigning numerical values of 0 for white, and 1 for yellow. The average colors are indicated numerically as well as by the darkness of shading in each box.

Figure 9. Four most-parsimonious trees obtained from a cladistic analysis of RAPD markers.

Figure 10. Strict consensus of four most-parsimonious trees obtained from a cladistic analysis of RAPD markers.

Figure 1

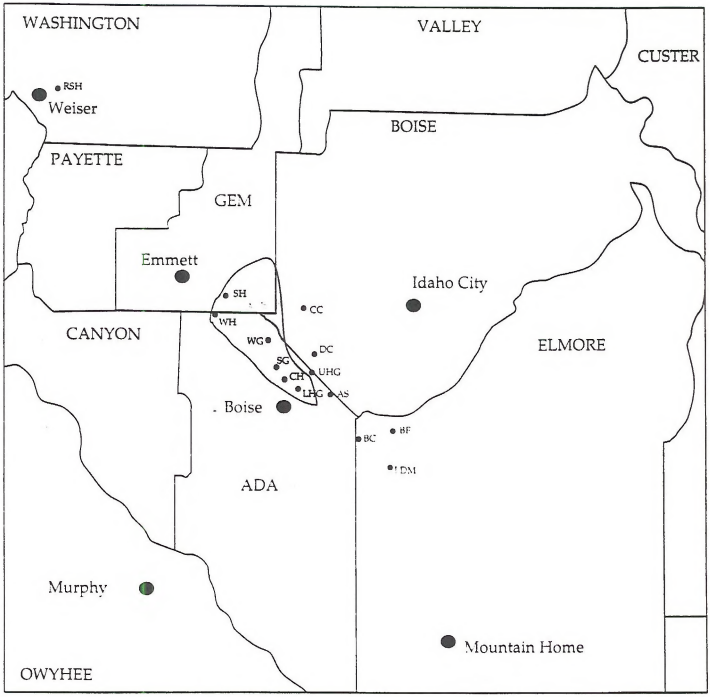

WASHINGTON
VALLEY
CUSTER
RSH
Weiser
BOISE
PAYETTE
GEM
Emmett
SH
CC
Idaho City
WH
CANYON
WG
DC
ELMORE
SG
UHG
CH
LHG
AS
Boise
BC
BF
IDM
ADA
Murphy
Mountain Home
OWYHEE

Figure 2

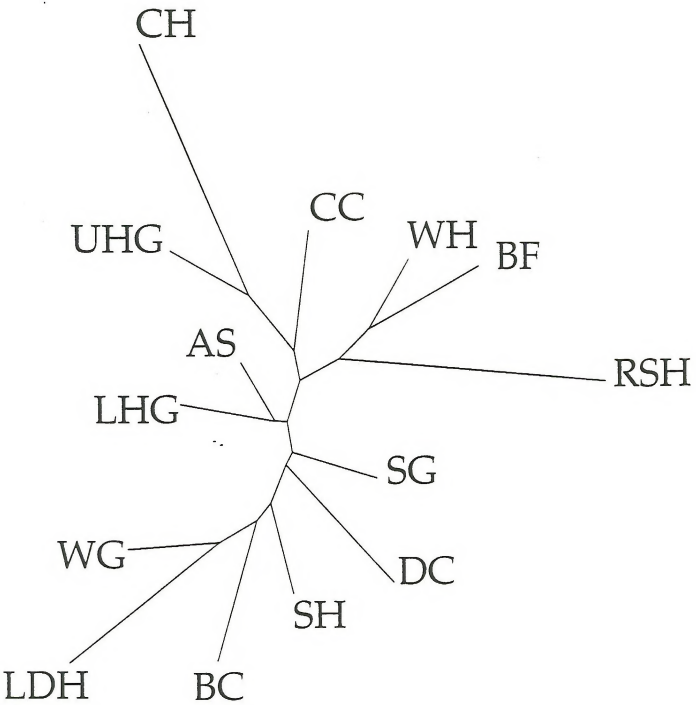


Figure 3: Frequency of Tepal Denticulations

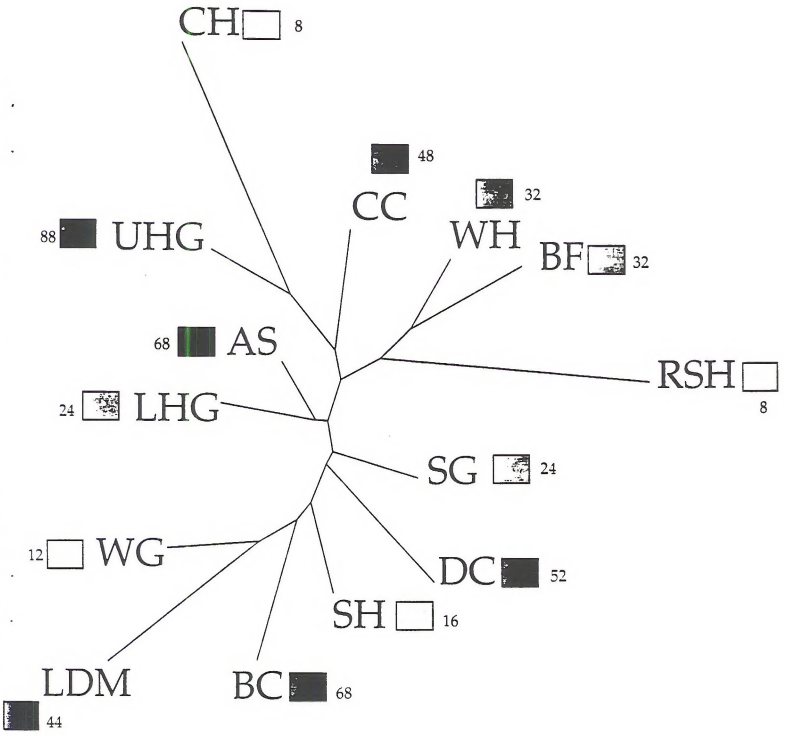


Figure 4: Scape Length

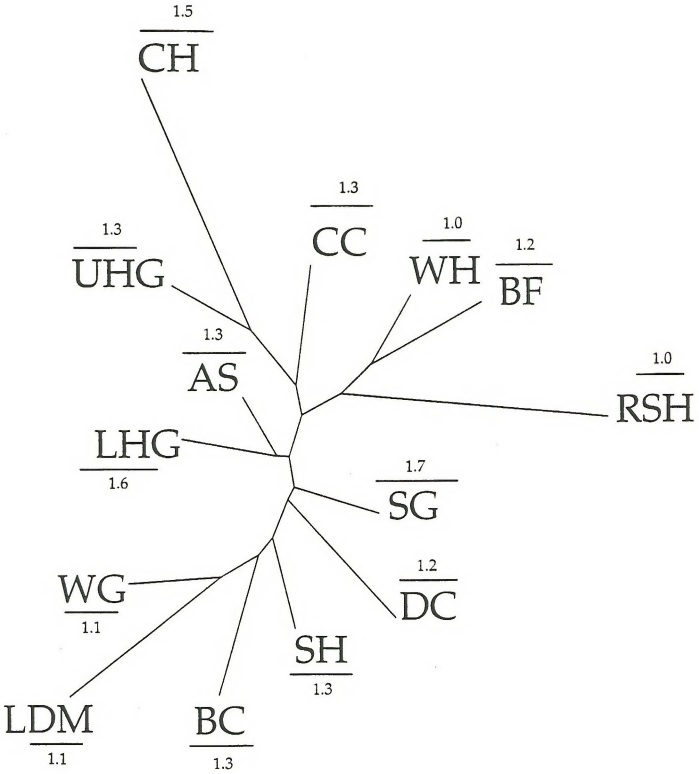


Figure 5: Bulb Depth

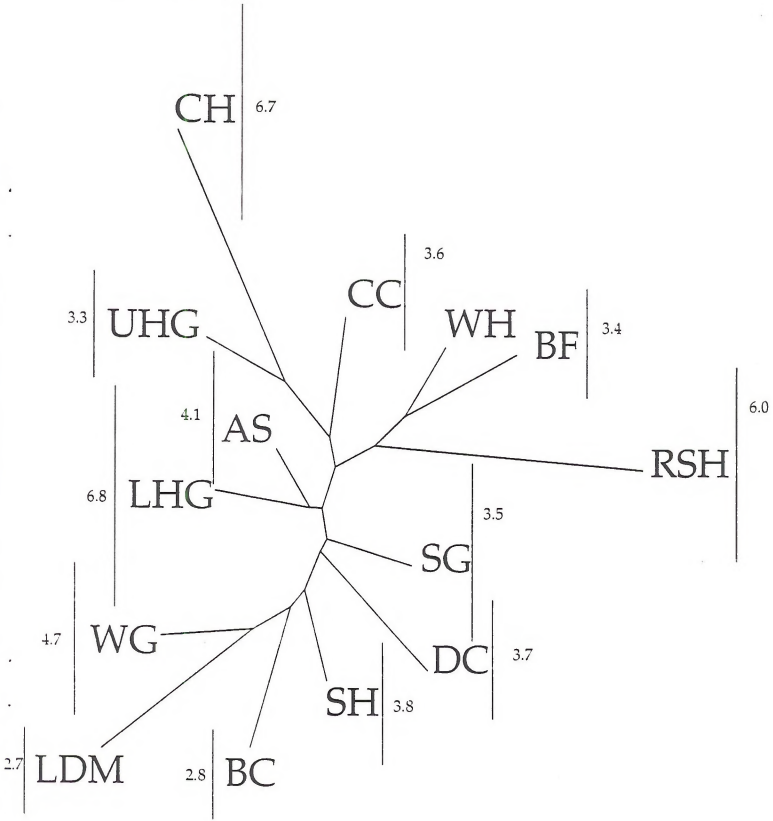


Figure 6: Tepal Color

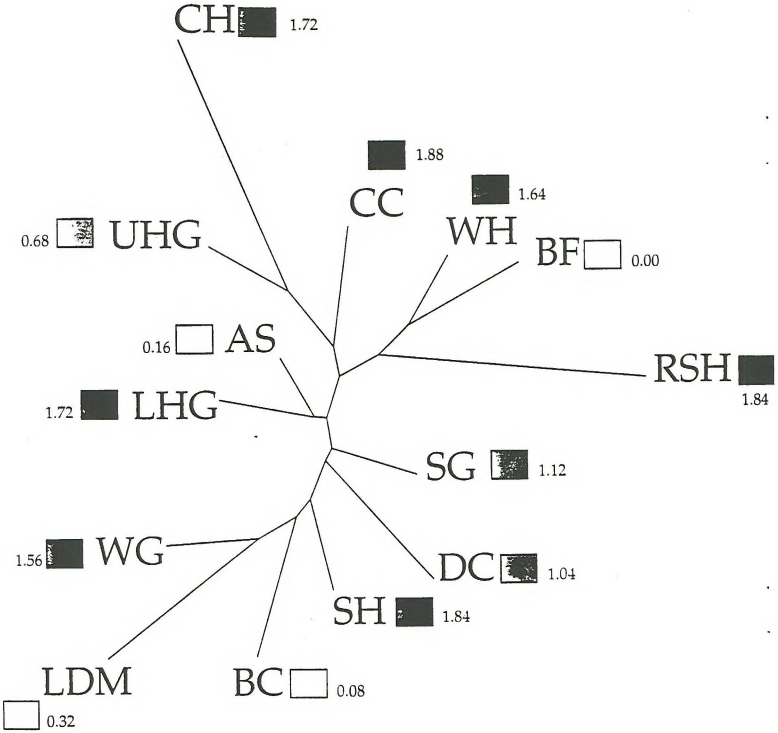


Figure 7: Anther Color

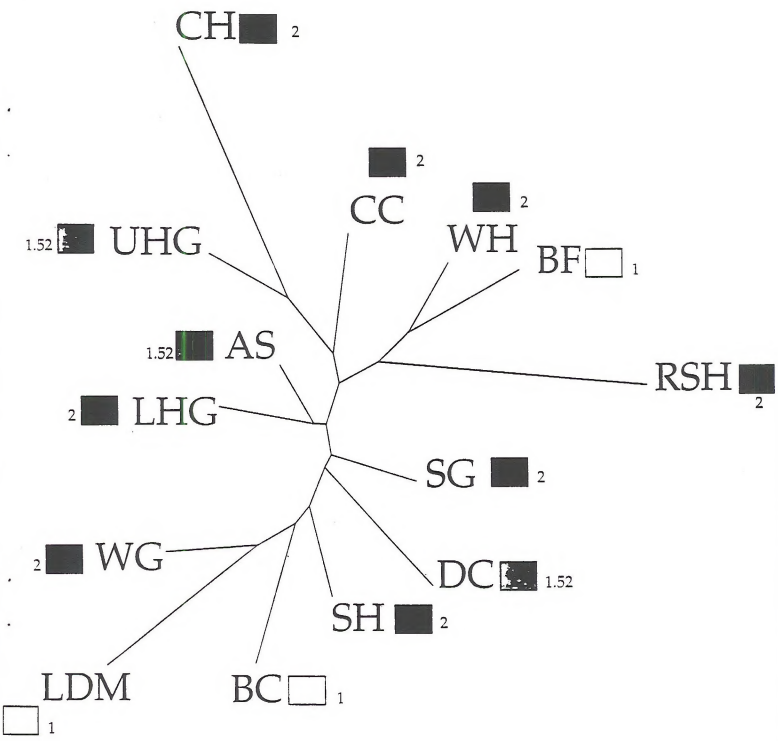


Figure 8: Pollen Color

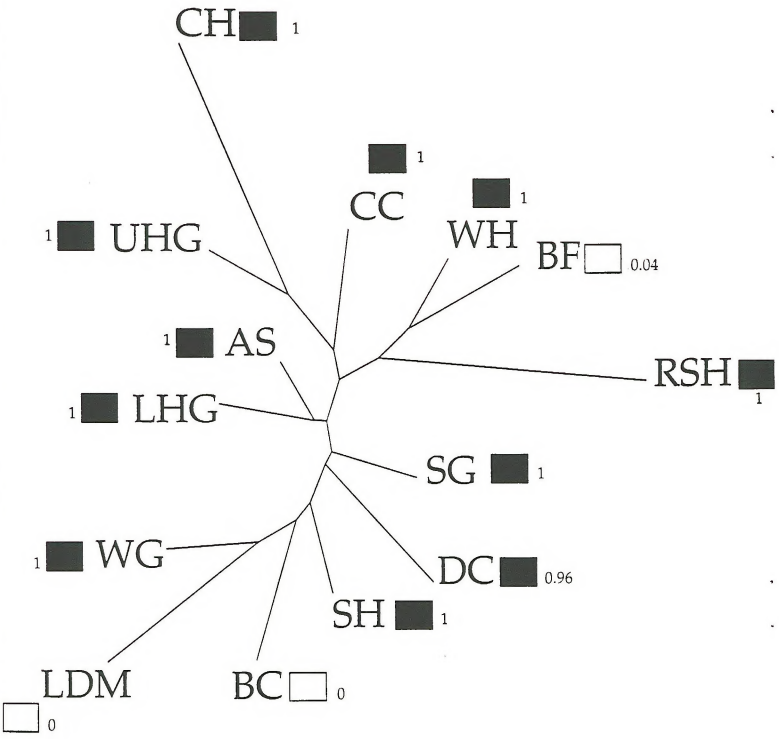
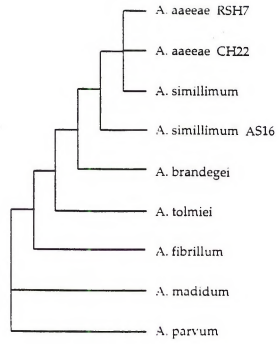
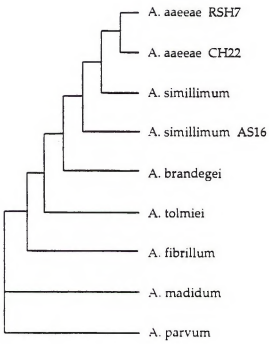


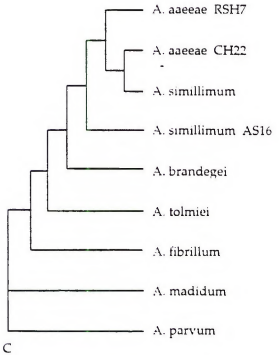
Figure 9



A



B



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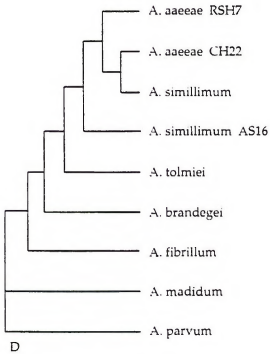
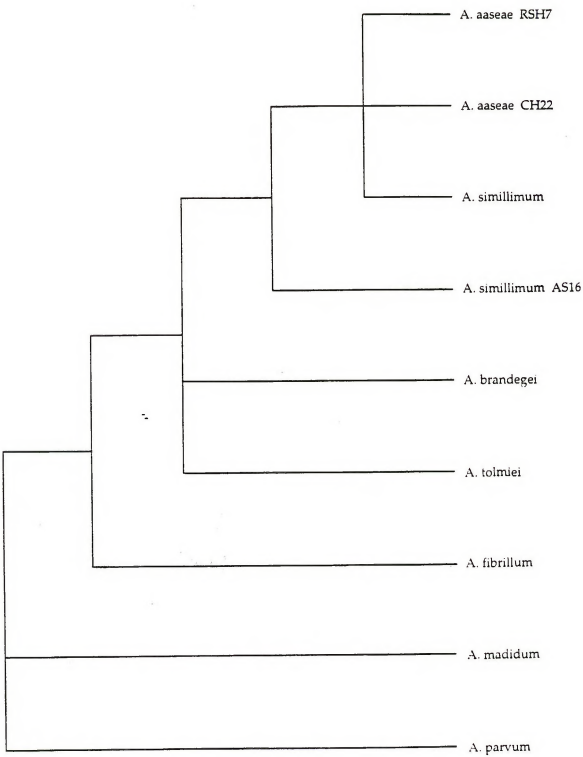


D

Figure 10


A. aaseae RSH7
A. aaseae CH22
A. simillimum
A. simillimum AS16
A. brandegei
A. tolmiei
A. fibrillum
A. madidum
A. parvum

NOT INCLUDED IN THIS PHOTOCOPY

(If you are interested in getting a copy of the Appendix data listed below, contact the Boise District of the Bureau of Land Management)

Appendix 1: Morphological features scored for all individuals used in this analysis. Denticulations on the topals are indicated as either present (1) of absent (0); topal color was classified as white, pale pink, pink, or dark pink. Scape length is measured in cm. Anther color was yellow, purple, or yellow and purple mottled. Pollen was either yellow or white.

Appendix 2: Presence or absence of loci for all 350 individuals sampled in this analysis. The loci are named in order forst by primer initials and then fragment size. The locus names are followed by each of the individuals with the order of loci in the same order.

Appendix 3: Gene frequencies for the loci surveyed in this analysis. The locus names are listed first, then followed by the frequencies of these loci for each population.

Appendix 4: RAPD markers used in the cladistic analysis of Allium in this study. The names of each marker are listed first, followed by the data for each species.

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BLM-ID-PT-95-007-1150